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A Long-Term Field Study of In Situ Bioremediation in a Fractured Conglomerate TCE Source Zone

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Bioremediation Field Study in Fractured Conglomerate

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ABSTRACT

An eight year bioremediation field study was conducted in a trichloroethene (TCE)-contaminated, highly indurated (i.e., hard), recharge limited (i.e., contains little water) conglomerate where common remediation strategies such as groundwater recirculation, and direct-push installation of a large well network, could not be used. A tracer test using isotopically distinct water from the Hetch Hetchy reservoir indicated remediation fluids mainly flowed through fractures and sand lenses in the conglomerate. This was confirmed during in situ bioremediation of the site, in which *Dehalococcoides* (from a bioaugmentation culture) and volatile fatty acids (from injection of lactate) were the most accurate indicators of transport between wells. Some contaminants were also displaced out of the area due to injection of tracer water. Despite these difficulties, dissolved contaminant mass decreased by an estimated 80% by the end of the test, reaching the lowest values ever recorded at this site. Furthermore, the persistence of ethene four years after bioaugmentation suggests the dechlorinating capacity of the remaining microbial community is comparable to the matrix diffusion of TCE into the dissolved phase.

KEY WORDS

bioremediation, groundwater, trichloroethene, rock, preferential flow, rebound, isotopes, tracer, *Dehalococcoides*

INTRODUCTION

The remediation of chlorinated solvents in low permeability sediments and fractured rock (Parker et al. 2008; Parker et al. 2010; Stroo and Ward 2010; SERDP – ESTCP 2011) continue to be a major challenge. One site that has attributes of both types of these geologies is the "T2" area at Lawrence Livermore National Laboratory Site 300 near Tracy, CA. This area consists of a trichloroethene (TCE)-contaminated, water-bearing conglomerate which is perched on claystone aquitard (Fig. 1). The conglomerate is heterogeneous and variably cemented, containing clays, friable sand lenses, and open fractures (Fig. 1). Stable, dissolved TCE concentrations of ~ 25 mg/L prevailed at this site for at least 15 years prior to the present work (historical data, not shown), and moderate soil concentrations (0.006 to 1.2 mg/kg) have been detected in soil samples (data not shown). However, no free product or "DNAPL" has been detected at this site. Therefore, it is likely that the clays and sands act as long-term sources of TCE, which are connected to preferential flow paths created by the fractures.

Additional site characteristics complicate its remediation. The conglomerate is recharge limited and variably saturated, with an average saturated thickness of only 0.9 m (3 ft) – 1.5 m (5 ft) (shaded/dark blue regions, Fig. 2). Wells are readily pumped dry, making water extraction on a continuous basis impractical. The conglomerate is also highly indurated, typically requiring more than 50 blow counts to advance a 15 cm (6 in) split spoon sampler. As a result, attempts to install wells with direct push methods have failed at this site. Thus, strategies that require the installation of a large number of wells to spread remediation fluids over a large area, such as re-circulation cells (Steffan et al. 1999; Ellis et al. 2000; Major et al. 2002; Dybas et al. 2002; Lendvay et al. 2003; Scheutz

et al. 2008) and fracturing methods (Strong et al. 2004; Christiansen et al. 2010; Scheutz et al. 2010), are also impractical at this site.

The T2 area is not unique. Many other sites within Lawrence Livermore National Laboratory Site 300 contain water-bearing zones of limited volume, with persisting TCE concentrations. Therefore, the overall objective of this work was to conduct a long-term, pilot scale, field study of in situ bioremediation at the T2 area, to assess the efficacy of this technology at Site 300, and determine design parameters for up-scaling, if successful. The specific tasks were to:

- *Perform a constant head tracer test* to hydraulically characterize the source area, using an isotopically distinct drinking water as a conservative tracer;
- *Perform an in situ bioremediation test*, in which periodic additions of sodium lactate, plus a single addition of a bioaugmentation culture were made to the source area, and their effect on redox parameters and TCE dechlorination were monitored;
- *Perform a rebound test,* in which the site was monitored for an additional 1.5 years after sodium lactate injection was discontinued; and
- *Evaluate the performance of bioremediation,* by tracking long term trends in the contaminant concentrations, and by estimating the mass of dissolved contaminants and dechlorination products before, during, and after sodium lactate injection and bioaugmentation.

To support these tasks, multiple data sets (including hydraulic, geochemical, isotopic, molecular biological, and contaminant data) were collected.

SITE DESCRIPTION

Geology. As summarized above, the site consists of a heterogeneous, anisotropic, variably cemented conglomerate of limited aerial extent, perched above a silty claystone aquitard (Fig. 2). The conglomerate typically has 0.9 m (3 ft) – 1.5 m (5 ft) of saturated thickness and a hydraulic gradient of 0.05 m/m – 0.1 m/m (based on over 20 years of monitoring data, not shown), depending on seasonal rainfall (shaded/dark blue regions, Fig. 2). Although the overall bulk hydraulic conductivity is low (10^{-4} cm/sec - 10^{-6} cm/sec, based on slug tests, data not shown), friable sand lenses and open fractures (Fig. 1) represent complex, three dimensional networks at the source area scale capable of transmitting flow. Given these characteristics, the conglomerate is more accurately characterized as a "water-bearing zone" rather than a typical, high-yielding aquifer.

The well field used for the test is shown in Fig. 2a. Well W-1824 was used as the single injection well for both the tracer and bioremediation tests (below). All other wells functioned as performance monitoring wells, except well W-1825, which received bioaugmentation culture during the bioremediation test.

Biogeochemistry. Historic site data collected over 15 years indicate the groundwater contained in the conglomerate is aerobic, with near-saturation O_2 concentrations, elevated levels of nitrate ($\geq$ 100 mg/L NO_3^-) and sulfate (~80 mg/L SO_4^{-2}), and relatively stable TCE concentrations (~ 25 mg/L). Moderate concentrations of TCE (0.006 to 1.2 mg/kg) have been detected in soil samples collected during previous site characterization activities. There is little evidence of anaerobic TCE biodegradation, except for occasional detection of small amounts of 1,2-dichloroethene (DCE) (< 2 µg/L). This is consistent with a previous molecular biological study (Miller et al. 2007),

which found *Dehalococcoides* were absent in groundwater from this site, and a separate microcosm study, in which dechlorination stopped at DCE after four months of incubation with various substrates (data not shown).

METHODS

Overall experimental approach. This field study was conducted in three parts: a tracer test, an in situ bioremediation test, and rebound test. A timeline of major event is summarized in Table 1.

Constant head tracer test. A constant head tracer test (Reynolds et al. 1983) was performed using an isotopically distinct water (see **Tracer selection and detection**, below). Tracer water was maintained in the injection well (W-1824; Fig. 2) at a constant elevation of approximately 3 m (10 ft) above the pretest water level, by interlocking a valve delivering the tracer water to a float switch hung in the injection well at a given elevation. This was done purposefully to hydraulically stress the subsurface and increase the local groundwater gradient between the injection well and nearby performance monitoring wells.

In situ bioremediation test. After the tracer test, fluid addition was discontinued for almost one year (day 491-817, Table 1). Then from days 818-1,434, tracer water was injected again into W-1824, at a constant elevation of approximately 6 m (20 ft) above the pre-test water level to further increase the local hydraulic gradient. During this time, periodic additions (20 L – 40 L) of sodium lactate (60% w/w, JRW Bioremediation, Lenexa, KS) were also made to well W-1824 (Table 1).

The site was bioaugmented once on day 1,294 (Table 1) with the addition of 10 L of KB-1® bioaugmentation culture (SiREM, Guelph, Ontario, Canada) to well W-1825. The injection well W-1824 was not bioagumented because the TCE concentrations there were quite low, due to dilution created by the preceding tracer test. A single addition of lactate was also made to well W-1825 just prior to bioaugmentation (day 1,281, Table 1) to support dechlorination, since volatile fatty acids (VFAs) had not yet been detected in downgradient wells (see **Bioremediation test: Redox indicators**, below).

Rebound test. Tracer water and sodium lactate injection were discontinued on day 1,434 (Table 1), and the site was monitored for an additional 600 days to assess contaminant rebound. Then, all activities were concluded on day 2,931 (Table 1).

Tracer selection and detection. Bromide was initially proposed as a tracer, but was not used due to regulatory concerns that it would persist above the Suggested No-Adverse Response Level of 2.3 mg bromide/L (Cal EPA – SWRCB 2013).

Therefore, local drinking water was used as a conservative tracer. The local drinking water in this part of California comes (via aqueduct) from the Hetch Hetchy Reservoir (HHR) in Yosemite National Park, California. HHR water is derived primarily from snowmelt in the Sierra Nevada Mountains, and therefore has a "lighter" isotopic signature than the site's groundwater, which is derived from precipitation at much lower elevations (Faure, 1986; Coplen, 1993). The isotopic signatures of oxygen and hydrogen were quantified in terms of $\delta^{18}O$ and $\delta^{2}H$ values (in units of per mil), which are defined as (Faure 1986):

$$\delta^{18}\mathrm{O} = \left(\frac{\left(\frac{^{18}\mathrm{O}}{^{16}\mathrm{O}}\right)_{\text{sample}} - \left(\frac{^{18}\mathrm{O}}{^{16}\mathrm{O}}\right)_{\text{VSMOW}}}{\left(\frac{^{18}\mathrm{O}}{^{16}\mathrm{O}}\right)_{\text{VSMOW}}} \right) \times 10^3 \quad (1)$$

196

197

$$\delta\ ^{2}\mathrm{H} = \left(\frac{\left(\frac{^{2}\mathrm{H}}{^{1}\mathrm{H}}\right)_{\text{sample}} - \left(\frac{^{2}\mathrm{H}}{^{1}\mathrm{H}}\right)_{\text{VSMOW}}}{\left(\frac{^{2}\mathrm{H}}{^{1}\mathrm{H}}\right)_{\text{VSMOW}}} \right) \times 10^3 \quad (2)$$

198

199 where VSMOW is Vienna Standard Mean Ocean Water (Gonfiantini 1978).

200

201 By collecting water samples throughout the test, the shift of the isotopic signatures away
202 from the site's original signature, and toward the signature of HHR water, became a
203 means to track the movement of the HHR water throughout the subsurface. Assuming
204 the isotopic signature in a given sample is the result of simple mixing between HHR
205 water and site groundwater, a plot of δ^{2}H vs. δ^{18}O can be made (see Fig. 5a), and the
206 fraction of HHR water present can be calculated by:

207

$$\text{tracer fraction}(t) = \sqrt{\frac{\left(\delta^{18}\mathrm{O}_{pt} - \delta^{18}\mathrm{O}(t)\right)^2 + \left(\delta^{2}\mathrm{H}_{pt} - \delta^{2}\mathrm{H}(t)\right)^2}{\left(\delta^{18}\mathrm{O}_{pt} - \delta^{18}\mathrm{O}_{HHR}\right)^2 + \left(\delta^{2}\mathrm{H}_{pt} - \delta^{2}\mathrm{H}_{HHR}\right)^2}} \quad (3)$$

208

209 where $\delta^{18}\mathrm{O}_{\mathrm{pt}}$ and $\delta^{2}\mathrm{H}_{\mathrm{pt}}$ are pre-test (pt) oxygen and hydrogen isotopic signatures,
210 respectively; $\delta^{18}\mathrm{O}(t)$ and $\delta^{2}\mathrm{H}(t)$ are oxygen and hydrogen isotopic signatures in a sample

211 collected at time *t*, respectively; and HHR signatures were $\delta^{18}O_{HHR}$ = -13.7‰ and $\delta^{2}H_{HHR}$
212 = -101.5‰.

213 $\delta^{18}O$ values were measured using the CO_2 equilibration method (Epstein and
214 Mayeda 1953) on either a VG Prism III or GV Instruments IsoPrime (now Elementar
215 Americas Inc.) isotope ratio mass spectrometer, with an analytical precision of ± 0.1 ‰.
216 $\delta^{2}H$ values were determined using three techniques: the offline zinc reduction method
217 (Coleman et al. 1982), with an analytical precision of ± 1 ‰; the catalyzed H_2 gas-water
218 equilibration "Hokko bead" method (Coplen et al. 1991), with an analytical precision of
219 ± 2 ‰; and the automated chromium reduction method (Morrison et al. 2001), with an
220 analytical precision of ± 0.5 ‰. Samples with high (~20 ppm) TCE concentrations gave
221 spurious results when analyzed with the Hokko method, suggesting that hydrogen present
222 in TCE and other chlorinated solvents may have exchanged with the H_2 gas during
223 equilibration. To solve this problem, selected samples were pre-treated with
224 approximately 1 g of granular activated carbon to sequester the chlorinated solvents, and
225 re-analyzed with both the Hokko bead and chromium reduction methods. The resulting
226 $\delta^{2}H$ signatures agreed to within the analytical precision of the two methods.

227 **Detection of dechlorinating bacteria.** The movement of the bioaugmentation
228 culture was tracked by detecting the presence (or absence) of the vinyl chloride (VC)
229 reductase gene *vcrA*, which codes for the reductase responsible for dechlorination of VC
230 to ethene in *Dehalococcides* sp. (Müller et al. 2004). The *vcrA* gene was a good target
231 for the present test because it is present in the consortium comprising the KB-1® bioaugmentation culture (Waller et al. 2005), and was absent at this site prior to
232 bioaugmentation (Miller et al. 2007).
233

DNA was extracted from water samples (0.5-1.0 L) by centrifugation at 3,220 x g. The pellet was suspended in 8.5 mL of a solution containing 10 mM Tris-HCl, 1 mM EDTA, at pH 8.0. The suspended pellet was subjected to three freeze-thaw cycles of freezing in liquid nitrogen for 5 min and thawing at 65°C for 5 min. Proteinase K (45 µl of 20 mg/mL) and 10% (w/v) sodium dodecyl sulfate (450 µl) were added, mixed thoroughly, and incubated for 1 hr at 42°C. DNA was extracted from the sample as described previously (Yeager et al. 2004). The resulting DNA pellet was dissolved in 100-200 µl of a sterile solution containing 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 and used for the polymerase chain reaction (PCR) analyses outlined below.

A PCR primer set was designed to amplify a 260 bp portion of the *vcrA* gene found in *Dehalococcoides* sp. strain VS (Genbank accession AY322364.1) using Primer3 (v. 0.4.0) software (Rozen and Skaletsky 2000). A BLASTn search revealed that the amplicon sequence is unique to *Dehalococcoides* species. Primer sequences were (vcrA.F) 5'-GATGCAGAGTGGGTTATTCC-3' and (vcrA.R) 5'-CCTGTTCTACCCTGTTCACC-3'. Ten to 100 ng of DNA extracted from the well water samples was used as template in PCR. Each 50 µL PCR mixture contained sample DNA plus 2.5 mM $MgCl_2$, 250 µM of each deoxynucleotide triphosphate, 35.0 µg bovine serum albumen, 10.0 µL 5X GoTaq reaction buffer, and 1.25 U GoTaq Flexi DNA polymerase (Promega, Madison, WI). PCR assays were conducted using the following cycling conditions: 2 min at 95°C followed by 35 cycles of 95°C for 45 seconds, 52°C for 30 seconds, and 72°C for 30 seconds. This was followed by incubation at 72°C for 5 minutes.

Amplified PCR products were analyzed by electrophoresis through agarose gels

containing 2.0% (wt/vol) SeaKem® GTG agarose (CAMBREX BioScience Rockland Inc., Rockland ME) in 1X TAE buffer (40 mM Tris-Acetate, 1 mM EDTA, pH 8.0). Electrophoresis was for 60 minutes at 80 volts. Amplicon fragments were visualized by ethidium bromide staining and sizes were estimated by comparison to DNA fragments in Low DNA Mass™ Ladder DNA molecular weight standards (Life Technologies/Invitrogen, Carlsbad, CA).

Contouring, water volumes, and dissolved mass estimates. Iso-concentration contours for tracer fraction, TCE, and dechlorination products (DCE, VC, and ethene) were calculated with EarthVision 7.5 (Dynamic Graphics Inc., Alameda, CA). Contours were created for a 15.2 m (50 ft) x 15.2 m (50 ft) treatment zone (dotted line, Fig. 2a), whose size and location were chosen to encompass the maximum extent of tracer travel observed (see **Tracer test: well connectivity and travel times,** below).

The volume of water between contours was estimated from a three dimensional geospatial model of the site (also created in Earthvision 7.5), bounded on the top by the water table, on the bottom by the claystone aquitard, and on the sides by treatment zone (dotted line, Fig. 2a). Surfaces defining the water table and aquitard were interpolated using water elevation data and site characterization data, respectively. Then, the water volume was estimated by multiplying the subsurface volume between contours by an effective porosity of 0.2 (determined separately, data not shown). Lastly, the masses of dissolved contaminants and ethene between adjacent contours were estimated by multiplying the geometric mean of the two contour values and the volume between the two contours, as described above. (For regions above the highest contour, the concentration was taken as the geometric mean of the contour value and the maximum

value of a given data set.) Lastly, the masses between adjacent contours were summed to estimate the total mass of a given compound.

Miscellaneous analytical and field methods. TCE, DCE, and VC were quantified from groundwater samples (40 mL; collected in "VOA" vials with inert caps) by a commercial laboratory (BC Labs, Bakersfield, CA) using USEPA Method 601. Ethene, ethane, and methane (LHA Method AM20GAX), and selected VFAs (VFA Method AM23G) were measured by a separate commercial laboratory (Microseeps Inc., Pittsburgh, PA) using provided vials and preservatives. Inorganic constituents and selected metals were analyzed by a commercial laboratory (BC Labs, Bakersfield, CA) using various EPA methods, including USEPA Methods 200.7, 200.8, 300, and SM23B. Water levels and oxidation reduction potentials were measured by separate, dedicated, "in-hole" transducers (Instrumentation Northwest, models PS-9800 or DL2; Yellow Spring Instruments, model 600XLM; respectively).

RESULTS

Tracer test: injection rate and water elevations. A total of 23,894 L (6,312 gal) of HHR water was injected during the tracer test, from days 0-189. The injection rate reached a steady state value of 26 gal/day after approximately 75 days of injection (Fig. 3a).

Groundwater elevations of all wells during the tracer test are shown in Fig. 4. The constant head approach taken during the tracer test can be seen in Fig. 4a, which shows the nearly constant elevation maintained in the injection well (W-1824) by addition of HHR water from days 0-189. Water levels in downgradient monitoring wells

303 increased within a few hours of the start of HHR injection (Fig. 4b), reached a maximum
304 of approximately 2 ft during HHR injection (Fig. 4a), and returned to pre-test elevations
305 by the end of the tracer test (day 491, Fig. 4a).

306 **Tracer test: well connectivity and travel times.** Results of tracer monitoring
307 are shown in Fig. 5. Fig. 5a shows the raw isotopic data from one well, W-1825, as an
308 example of the data collected. The isotopic signatures in this well generally fall on a line
309 between the pre-test and HHR water signatures, supporting the assumption that the
310 signature at any point in time is the result of simple mixing between HHR water and site
311 groundwater. Then, the fraction of tracer in each well was calculated from Eq. 3, using
312 the δ^{2}H and δ^{18}O values from a particular sampling event (Fig. 5b). Lastly, the tracer
313 fraction data from all wells from a given sampling period were contoured. Fig. 5c shows
314 contours resulting from data collected on day 189, when the maximum coverage was
315 achieved.

316 This analysis yielded two results. First, most wells (W-T2, W-1825, and W-1833)
317 were hydraulically connected to the injection well (Fig. 5c). However two wells, W-T2A
318 and W-T2D, were clearly outside the influence of the injection well (Fig. 5c). This is
319 shown in greater detail for well W-T2D, whose tracer fraction showed no obvious trend
320 throughout the tracer test (squares, Fig. 5b), and most likely reflects background
321 variations in the isotopic signature of the site's water. Second, the travel times between
322 the injection and monitoring wells were readily determined. Data from Fig. 5c shows
323 that tracer reached the farthest monitoring well by day 189, or about 6 months. This
324 corresponds to an average groundwater velocity of 0.08 to 0.1 meters/day (0.25 to 0.33
325 ft/day), achieved under the condition of constant head injection.

Bioremediation test: Injection rate and biofouling. A total of 43,149 L (11,403 gal) of HHR water and 696 L (184 gal) of 60% sodium lactate were added to the injection well (W-1824) throughout the bioremediation test (days 818-1,434; Table 1). (These are in addition to the 23,894 L HHR water added during the tracer test.) The injection rate decreased sharply from an initial rate of 76 gal/day, to a steady state rate of 8 gal/day by the end of the test (Fig. 3b), suggesting the injection well became biofouled over time. This was supported by frequent observation of a slime covering instrumentation inside the injection well, which required periodic cleaning.

Bioremediation test: Redox indicators. Slugs of sodium lactate were added to the injection well over a 616 day period, as indicated by the black hatch marks at the top of Fig. 6. Decreasing nitrate and sulfate concentrations (Fig. 6a-c), negative ORP values (Fig. 6a-c), and elevated methane concentrations (data not shown) during this period suggested the redox conditions in the subsurface were conducive to anaerobic dechlorination of TCE. However, no VFAs were detected in downgradient wells (Fig. 6d-f) after more than 500 days of periodic lactate addition. These initial observations suggested the biological activity that reduced nitrate, sulfate, and ORP levels was localized in the immediate vicinity of the injection well W-1824, and consumed a majority of the lactate and other VFAs before they could be transported downgradient.

Given the absence of VFAs in the subsurface, two slugs of sodium lactate were added to well W-1825 immediately before and after it was bioaugmented (red hatch marks, Fig. 6), to provide additional energy for dechlorinating bacteria (Fennell et al. 1997). This resulted in a spike of VFAs observed in the surrounding wells around day

348 1,400 (Fig. 6d-f). The spike in VFAs in well W-T2 was an important observation,
349 because typically this well is upgradient from well W-1825 (see Fig. 7c).

350 **Bioremediation Test: *Dehalococcoides* and ethene distributions.** As observed
351 in other field studies (Scheutz et al. 2008; Lu et al. 2006a; Lu et al. 2006b; Rahm et al.
352 2006; Van der Zan et al. 2010), detection of ethene correlated with the presence of
353 *Dehalococcoides* species. The *vcrA* gene indicative of *Dehalococcoides* was detected in
354 wells where ethene was present (W-1825, W-T2), whereas it was not detected in well W-
355 1833 where ethene was absent (Fig. 7a-b). Given the absence of *Dehalococcoides* at this
356 site prior to this study (Miller et al. 2007), the detection of the *vcrA* gene possessed by the
357 KB-1® consortium (Waller et al. 2005) after bioaugmentation strongly suggests
358 bioaugmenation was responsible for the observed ethene production.

359 The detection of ethene and *Dehalococcoides* in well W-T2 was unexpected,
360 because this well (blue line, Fig. 7c) was upgradeint of well W-1825 (pink line, Fig. 7c)
361 throughout much of the test, where bioaugmentation culture was added. However,
362 hydrograph data indicate the groundwater elevation was temporarily higher in well W-
363 1825 due to the addition of lactate and bioaugmentation culture (region inside dotted
364 lines, Fig. 7c). This transient shift in local groundwater gradient direction likely provided
365 the hydraulic potential to transport lactate, VFAs, and *Dehalococcoides* from well W-
366 1825 to well W-T2.

367 **Bioremediation and Rebound Tests: Performance evaluation.** The overall
368 performance of bioremediation was evaulated by considering long-term trends in the total
369 volatile organic compounds (TVOCs, defined as the sum of TCE, DCE, and VC in μg/L)
370 (Fig. 8a), and estimating the mass of individual dechlorination products within the

371 treatment zone (Fig. 8b). The TVOCs in all of the wells generally decreased throughout
372 the test (Fig. 8a). However, the wells separate into two groups: those whose TVOC
373 trends were largely unchanged during the test (wells W-1833, -T2A, and –T2D), and
374 those whose TVOCs decreased substantially by the end of the test (wells W-1824, -1825,
375 and -T2). Notably, the TVOCs in well W-T2 (+, Fig. 8a) decreased around day 1,500,
376 rebounded around day 2,000, and then decreased again at the end of the test, suggesting
377 the decrease in this well is a long-term affect of bioremediation. The decreases in wells
378 W-1824 and W-1825 (◊ and ○, Fig 8a) were expected because they received "clean"
379 HHR water and bioaugmentation culture, respectively.

380 The performance was also evaluated by estimating the masses of dechlorination
381 products in the treatment zone (Fig. 8b). Before any lactate or bioaugmentation culture
382 was added, TCE and DCE were the only contaminants present (day 784, Fig 8b). Then,
383 the amount of DCE increased after the beginning of lactate injection (day 1,244, Fig. 8b),
384 and then VC and ethene appeared after bioaugmentation (Fig. 8b). The maximum
385 amount of ethene was detected soon after bioaugmentation on day 1,581 (Fig. 8b),
386 equivalent to about 50% of the TVOC mass. By the end of the rebound test (day 2,931,
387 Fig. 8b), the dissolved TVOC mass was reduced by about 80% within the treatment zone
388 compared to the TVOC mass before the test, and the ethene mass was equivalent to about
389 one third of the TVOC mass. Dilution and displacement of contaminants out of the
390 treatment zone due to HHR injection accounts for some of the contaminant loss.
391 Displacement is difficult to estimate, but dilution, which is directly measured by tracer
392 fraction, was about 25% at the end of the rebound test (data not shown).

393 **Bioremediation test: metals.** Manganese and iron each reached maximum
394 concentrations of approximately 25 mg/L, but their values decreased and approached
395 background levels by the end of the test (data not shown). Contrary to observations with
396 other soils (McLean et al. 2006; He et al. 2010), neither arsenic nor any of the other
397 metals monitored increased in concentration throughout the test (data not shown).
398
399 **DISCUSSION**
400 Low permeability and complex subsurface hydraulics posed a major challenge to
401 the delivery of electron donor and dechlorinating bacteria at this site. During the tracer
402 test, water levels in all downgradient wells responded (i.e., began increasing) within
403 hours of starting tracer injection, but it took 3 months for tracer to reach downgradient
404 wells in detectable concentrations (Fig. 5). Some wells within the treatment zone
405 exhibited no evidence of tracer arrival at any time during this long-term test. Although
406 hydraulic pressure was transmitted quickly within the treatment zone under constant head
407 injection, the actual tracer transit times between wells varied over much longer time
408 scales, on the order of several months. These observations are consistent with the
409 interpretation that subsurface transport at the treatment zone scale (15.2 m (50 ft) x 15.2
410 m (50 ft)) is dominated by a three-dimensional network of fractures and friable sand
411 layers of varying interconnectivity interspersed within a nearly impermeable cemented
412 conglomerate (Fig. 1).
413 Complex flow patterns were also observed during the bioremediation test.
414 Despite adding lactate for 500 days into well W-1824, no VFAs were observed in any
415 downgradient wells (Fig. 6d-f). However, VFAs and *Dehalococcoides* were detected in

well W-T2 125 days after a small volume of lactate was added to W-1825 (Fig. 7). Although VFAs are not conservative tracers, their appearance in well W-T2 suggests they, like the HHR water, also flowed through preferential paths created by the fractures and sand layers (Fig. 1). These pathways result in variable transit times and transport lengths for remediation fluids within the test site. The general difficulty of remediating formations dominated by complex flow has been observed by others (Zheng and Gorelick 2003, and references therein).

The use of an isotopically distinct water as a tracer had advantages and disadvantages. The major advantage of this approach is that it provides a direct estimate of dilution. For example, a sample with a tracer fraction of 10% indicates approximately 10% dilution. Injecting water under constant head also had the advantage of increasing the local gradient and accelerating the transport of remediation fluids in a formation with a typically small gradient. Unfortunately, large volumes of tracer water were required to reach a detectable tracer signal. The natural, "background" fluctuation of the site groundwater's signature was approximately 5%, based on wells not influenced by injection (e.g., well W-T2D, Fig. 5b, squares). Thus, a truly detectable fraction was judged to be ≥10% (e.g., well W-1825, Fig. 5b, triangles), which required the injection of large quantities of HHR water to achieve (a total of ~ 67,000 L (17,700 gal) from the tracer plus bioremediation tests). The large volume of water injected then led to dilution and displacement of some TCE out of the treatment zone.

The only technically defensible measure of long term performance of any remediation technology is the continued reduction in contaminant mass concurrent with the persistence of benign end products. Estimating mass removal performance is

inherently difficult, because it requires detailed quantitative knowledge of processes that add (source terms, dissolution of free product, desorption and diffusion of contaminants from sediments), remove (abiotic degradation, biodegradation), and transport (hydraulic conductivity distribution and groundwater flow) contaminant mass in the subsurface. Mass flux measurements (ITRC 2010) are now commonly used to measure these processes at sites where closely-spaced, monitoring points can be readily installed using push technology. However, direct push methods were attempted at this site and failed, due to the highly indurated nature of the subsurface, so mass flux methods could not be used. Instead, we monitored TVOC trends in wells during a prolonged rebound test to observe the magnitude of contaminant rebound, and its trend over time, to help assess long term performance (Fig 8a). Furthermore, by using a volumetric approach, we were able to demonstrate a significant overall reduction in dissolved contaminant mass concurrent with ethene production.

CONCLUSIONS and MAJOR FINDINGS

The results of this study suggest that in situ bioremediation can be used to reduce contaminant mass in fractured conglomerate TCE source areas. The main difficulty is characterizing the three dimensional hydraulic connections between wells that influence delivery of remediation fluids and bioaugmentation cultures. The only practical was this can be done is to perform a pilot scale test and collect an interdisciplinary set of hydraulic, chemical, and microbiological data to understand the movement of remediation fluids in the subsurface. Our major findings were:

- Complex flow paths, most likely related to fractures and sand lenses within the conglomerate, dominated the subsurface hydraulic connectivity and travel times between wells at this site.

- Tracer injection in this type of geologic environment tends to be more influenced by "piston flow" than by dispersive mixing, resulting in some unavoidable plume displacement.

- A large quantity of isotopically distinct tracer water was required to produce a detectable signal of $\delta^{18}O$ and $\delta^{2}H$ in downgradient wells, resulting in some displacement of contaminants out of the treatment area.

- Detection of VFAs, *Dehalococoides*, and dechlorination products were the most accurate indicators of transport between wells. Other parameters, such as redox indicators (anions, ORP), hydraulic response, and tracer ($\delta^{18}O$ and $\delta^{2}H$) arrival were not reliable predictors of transport between wells, or where bioremediation fluids could be spread.

- The dissolved contaminant mass decreased an estimated 80% by the end of the test, reaching the lowest TVOC values ever recorded at this site. Based on the isotopic composition, roughly 25% of the groundwater remaining in the treatment zone at that time was HHR tracer.

- The presence and persistence of ethene four years after bioaugmentation suggests the dechlorinating capacity of the remaining microbial community is comparable to the diffusive flux of TCE into the dissolved phase.

ACKNOWLEDGEMENTS

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TABLE 1. Sequence of Events During the Tracer, Bioremediation, and Rebound Tests.

Day	Event
	Tracer Test
0	Began constant head HHR[a] injection into W-1824
	Began collection of lab samples and field data
189	Ended HHR[a] injection into W-1824
	Continued collection of lab samples and field data
491	Ended collection of lab samples and field data
	Bioremediation Test
818	Began constant head HHR[a] injection into W-1824
	Began periodic lactate addition into W-1824
	Began collection of lab samples and field data
1,281	Single lactate addition into W-1825
1,294	Bioaugmentation of W-1825
1,434	Single lactate addition into W-1825
	Ended all HHR[a] injection, lactate addition
	Rebound Test
1,434	Continued collection of lab samples and field data
2,931	Ended collection of lab samples and field data

[a] HHR = Hetch Hetch Reservoir water tracer

FIGURE CAPTIONS

Figure 1. A picture of the conglomerate and claystone units, exposed in an outcrop on a hillside approximately 100 m from the test site. The contact between the two units is indicated by the dotted line. The bulk of the conglomerate is impermeable, except for fractures and sand lenses (indicated) which readily conduct water.

Figure 2. Plan view of the test site (*a*), and a cross section along transect A-A' (*b*). The cemented conglomerate is the contaminated water-bearing zone that is the subject of this study. Dark blue area represents unstressed water levels (typically 0.9 m (3 ft) – 1.5 m (5 ft)) found at this site; light blue area represents water levels created during the test, due to injection of tracer water. Dotted line in (*a*) represents the effective treatment zone created by injecting fluids into well W-1824, and is the region in which contaminant and dechlorination product masses were estimated.

Figure 3. Injection rates of HHR tracer into well W-1824 during: (*a*) the tracer test, and (*b*) the bioremediation test. Note that during the tracer test, the water level in the injection well was maintained at constant elevation of about 3m (10 ft) above the pretest water table; during the bioremediation test, the water level was maintained at a constant elevation of about 6m (20 ft) above the pretest water table. Arrows indicate short time periods when the injection system was not operating due to maintenance.

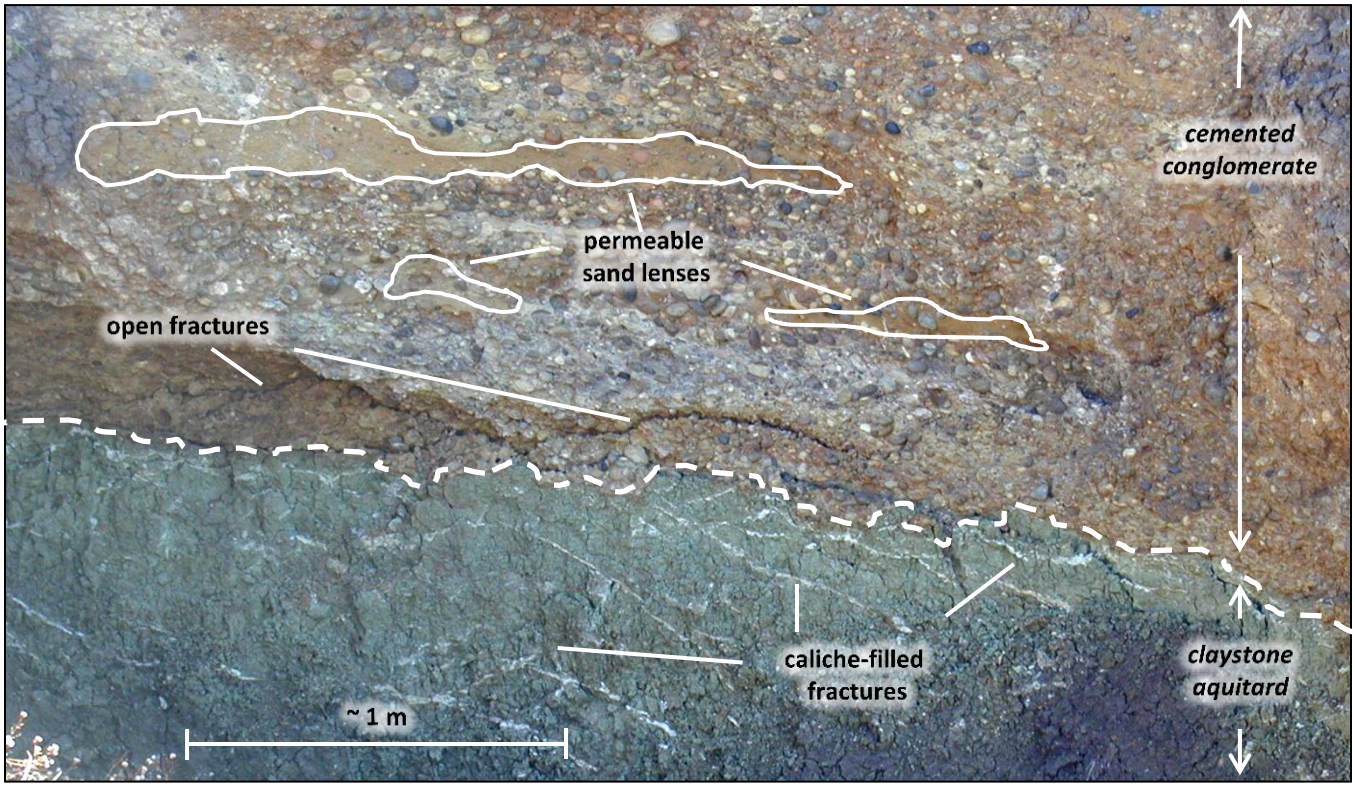
Figure 4. Groundwater elevations during the tracer test. Panel *a* shows elevations for entire test. Panel *b* is a close-up at the beginning of the test, highlighting the quick response (e.g., increase) in elevations soon after injection was started. Solid lines are water elevations from different wells measured semi-continuously by data-logging transducers. Symbols are water elevations from the same wells measured by hand, as a check on the accuracy of the transducer data.

Figure 5. Isotopic response of wellfield during the tracer test. Panel (*a*) is an example of raw data collected, showing δ^{2}H and δ^{18}O signatures in a single well (W-1825) observed at different times throughout the test. Panel (*b*) is an example of the range of well responses observed, comparing the tracer fraction in well W-1825 which responded strongly to tracer injection, with well W-T2D which was essentially unresponsive to tracer injection. Panel (*c*) shows extent of maximum tracer influence, observed on day 189.

Figure 6. Redox indicators measured in selected wells during the bioremediation and rebound tests. Anion and ORP concentrations are shown in panels *a-c*; VFA concentrations are shown in panels *d-f*. Indicated on each plot are: additions of sodium lactate into the injection well W-1824 (black hatch marks) and well W-1825 (red hatch marks); and a single addition of bioaugmentation culture to well W-1825 (closed/green arrow).

Figure 7. Distribution of ethene and *Dehalococcoides* during the bioremediation test. Panel *a* shows contours of ethene (μg/L) dissolved in groundwater on day 1,581 when the highest ethene concentration was observed. Panel *b* is a gel showing results of PCR amplification of the *vcrA* gene from selected wells on the same day. Panel *c* shows how the water elevation in the well that was bioaugmented (well W-1825) increased for a short period of time (region inside dotted lines), making transport of *Dehalococcoides* and VFAs to well W-T2 possible. In panel *c*, note that solid lines are water elevations from different wells measured semi-continuously by data-logging transducers; symbols are water elevations from selected wells measured by hand, as a check on the accuracy of the transducer data.

Figure 8. Evaluating the performance of bioremediation. Panel *a* shows long term trends in total volatile organic compounds (TVOCs; TVOCs = TCE + DCE + VC (μg/L) in dissolved phase). Panel *b* shows mass estimates for dissolved TCE and all dissolved dechlorination products on selected days throughout the bioremediation and rebounds tests. Note that the mass of TCE rebounds on days 2,037 and 2,261, but decreases again by the end of the test (day 2,931).



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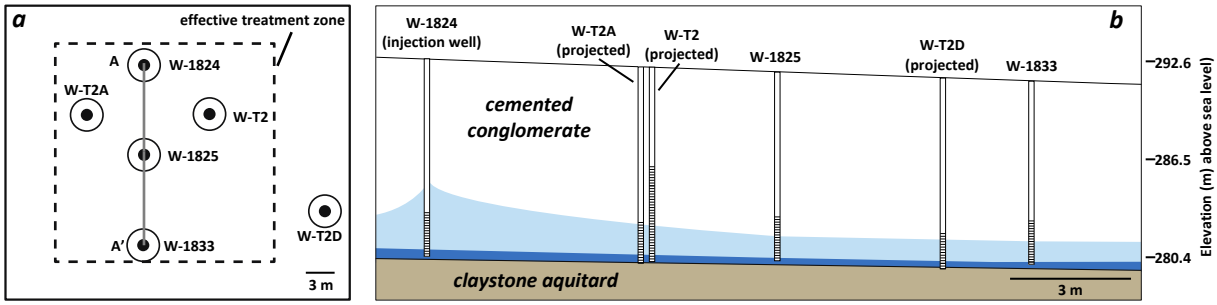

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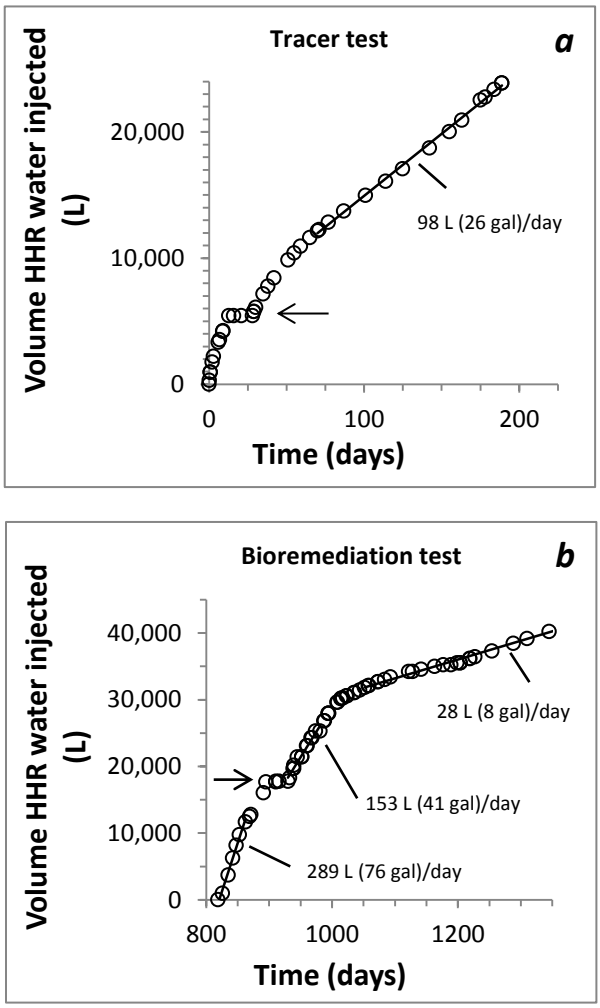

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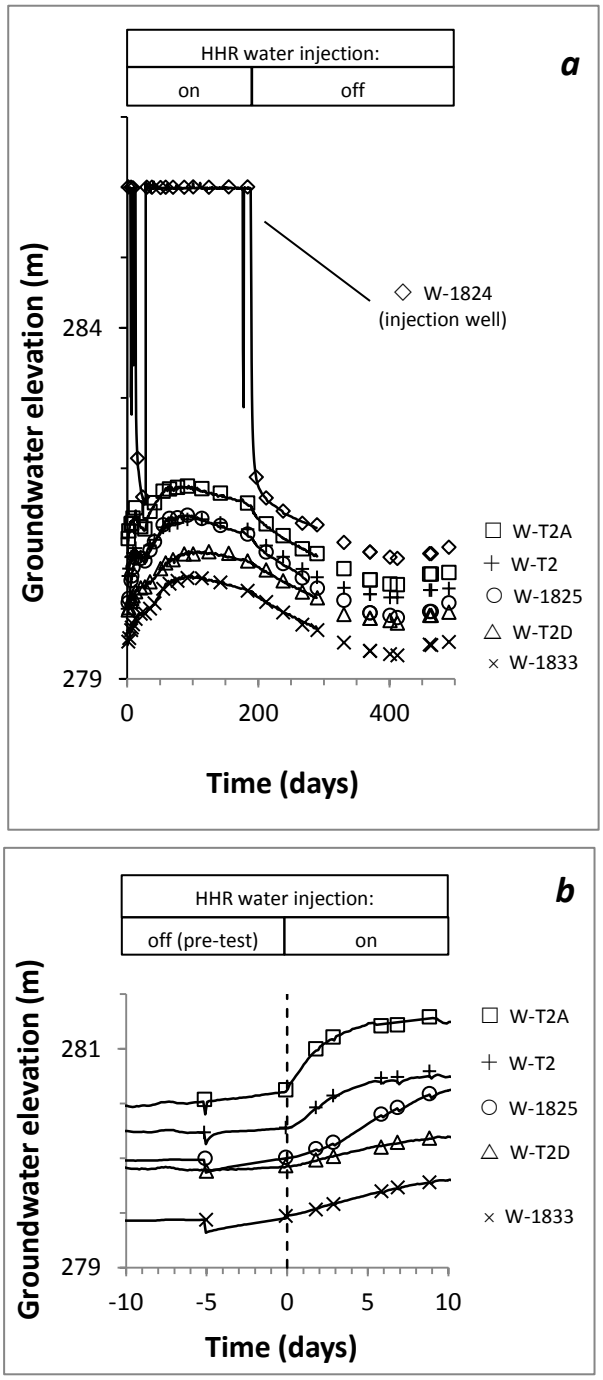

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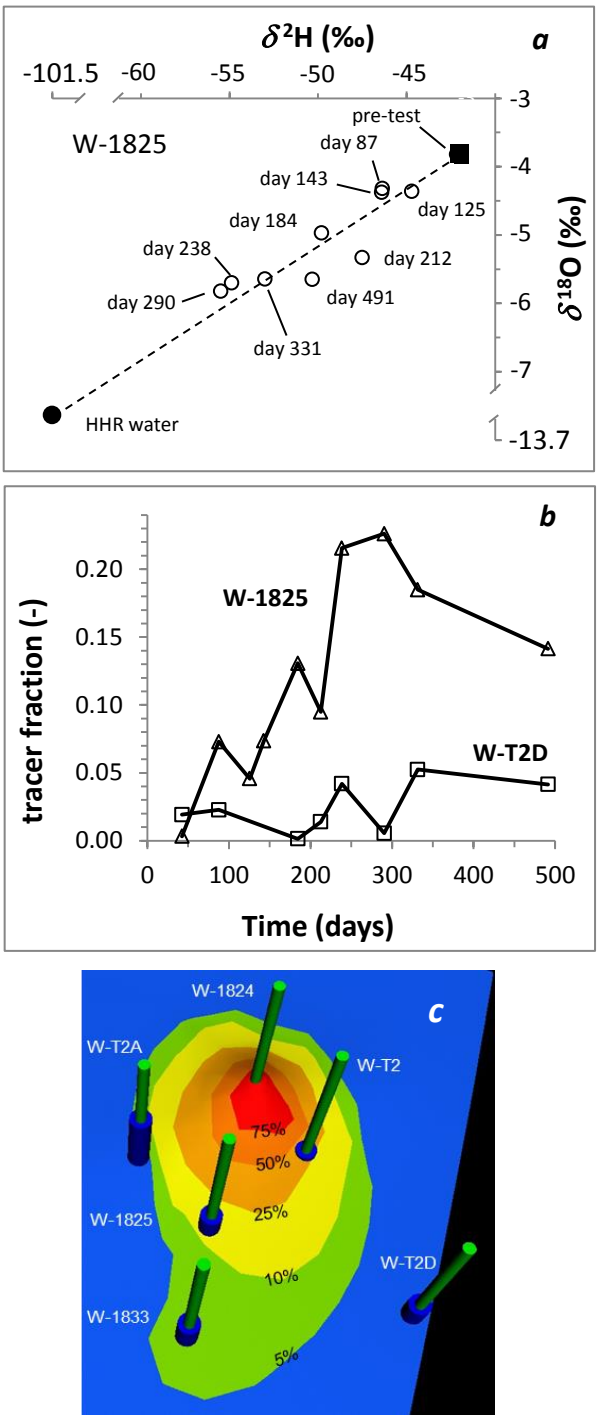

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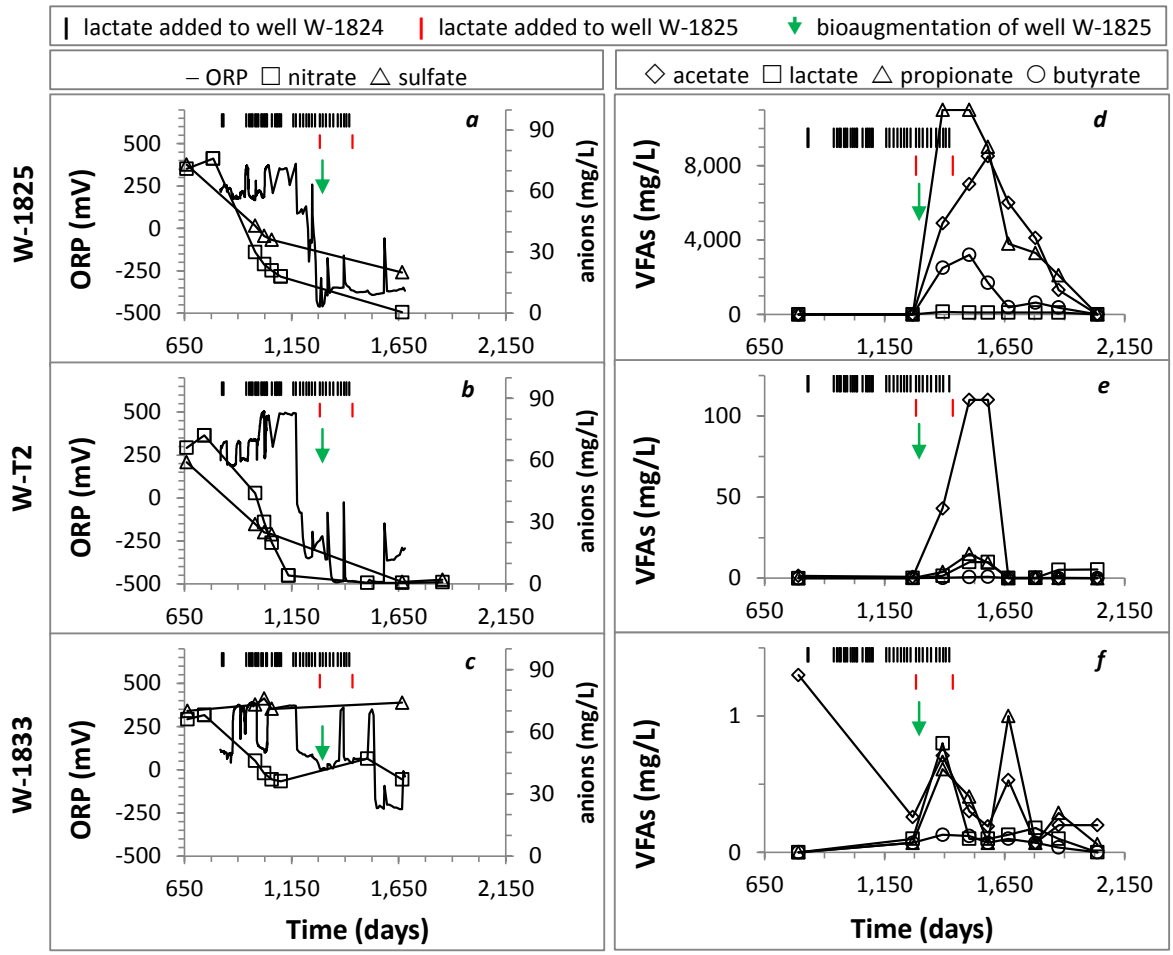

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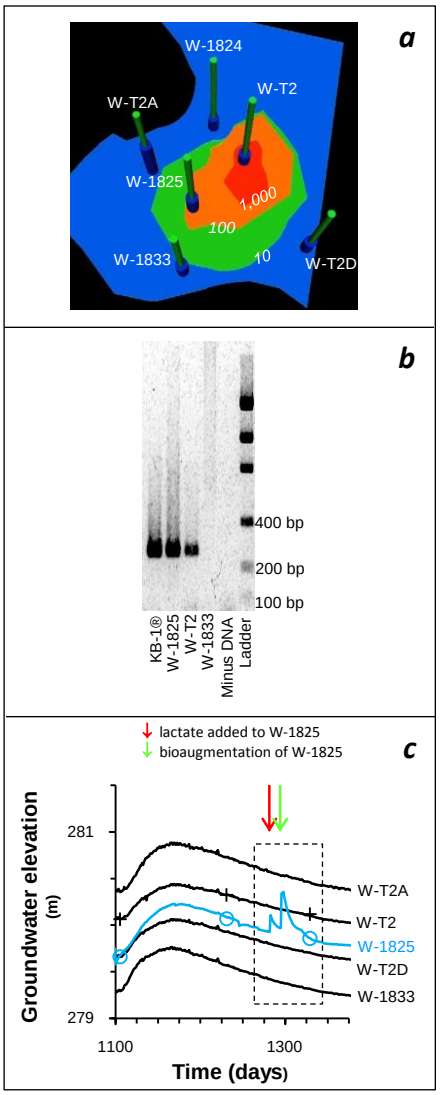

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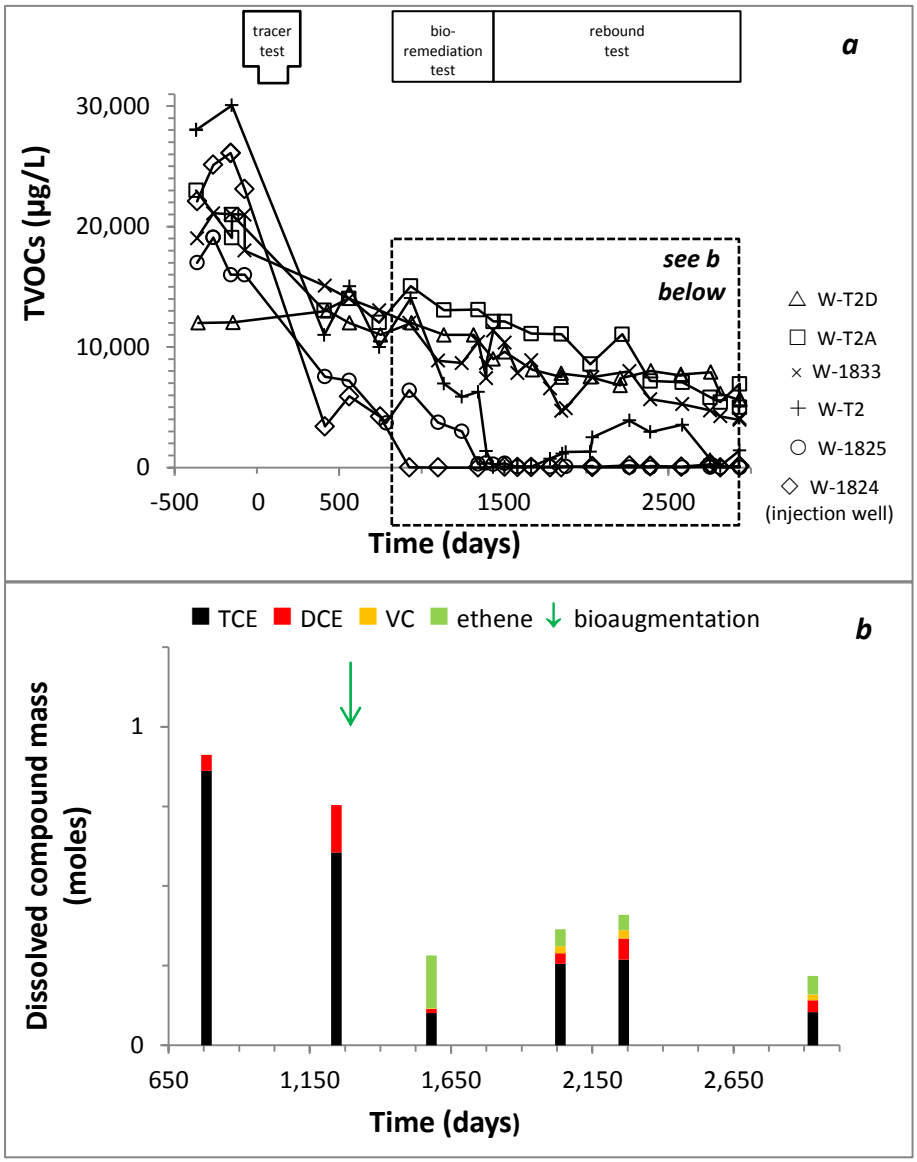

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